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Milk Lipases and the Association of Lipases with Casein Micelles studied by Gel-Filtration

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Most of the lipase activity in cows' milk is sedimented with the casein micelles by ultracentrifugation, but supernatant solutions containing about 70% of the activity are obtained by centrifuging milk containing 0·75 M-NaCl at 80000 *g* for 1 hr. Partial separation of the lipases in such preparations can be achieved by gel-filtration on columns of Sephadex G-200 equilibrated with 0·75 M-NaCl. Studies of substrate specificity of the fractionated enzymes indicate the presence of a more complex mixture than we hitherto supposed (Downey & Andrews, 1964, 1965).

Most of the lipase activity seems to be due to five enzymes whose molecular weights, as judged by their gel-filtration behaviour (Andrews, 1964), range from about 50000 to 130000. They have low substrate specificities, for they all hydrolyse tributyrin, triolein and milk-fat emulsions, and triacetin in solution. However, their separate identities are confirmed by the fact that the activity of the higher molecular weight enzymes towards triolein and milk fat, relative to their activity towards tributyrin, is appreciably greater than that of the lower molecular weight enzymes. Conversely, the activity of the latter enzymes towards triacetin, relative to their activity towards tributyrin, is greater than that of the former enzymes. Variations from milk to milk in the relative amounts of the five enzymes are reflected in the rates at which different milks hydrolyse the four substrates.

Reversible association of the lipases with the casein micelles was demonstrated when the enzyme preparations were subjected to gel-filtration in solutions of lower ionic strengths. In 0·1 M-NaCl the enzymes were eluted almost entirely with the micelles, whereas in 0·2 M-NaCl they were found in an asymmetric peak which emerged after the casein peak, and extended almost to the positions characteristic of the free enzymes.

The ability to associate with casein micelles seems to be a property of lipases, since when pancreatic lipase was admixed with a milk lipase preparation and subjected to gel-filtration in 0·1 M-NaCl it was eluted with the micelles, but when admixed with a preparation from which the casein had been removed by calcium precipitation (Bohren & Wenner, 1961) it was eluted in its normal position. Other enzymes, including wheat-germ esterase, showed little or no tendency to associate with the micelles. Since lipases attack emulsified substrates, substrate-binding at an interface and association with casein micelles might be similar phenomena. The possibility that the complete micellar structure is necessary is suggested by the failure of pancreatic lipase to associate with purified α-, β- or κ-caseins.

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